HISTOLOGICAL COMPARISON OF THE B16 MELANOMA AND ITS F1 VARIANT

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SUMMARY

The B16 malignant melanoma and its F1 variant line were described and compared in syngeneic C57BL/6 mice at short and long term periods after transplantation. No observable differences in gross or histological features were noted. In both lines, liver and lung metastasis was confirmed and kidney colonization proposed.

INTRODUCTION

In 1954, the B16 melanoma arose spontaneously in the skin at the base of the ear of a C57BL/6 mouse, an inbred strain [1]. The tumor is found to metastasize to the lungs, liver and spleen with fatality standing at 100% and mouse death usually occurring 3–5 weeks after transplantation [2]. Tumor histology, after trocar passage, has been previously discussed [2,3].

The B16-F1 variant, chosen for its ability to colonize the lungs, was first established, by Fidler, through the process of in vivo selection [4,5]. Initially, B16 tumor cells were intravenously injected into C57BL/6 mice. Pulmonary metastases that appeared after 3 weeks were removed and grown in tissue culture as a continuous line, the B16-F1. Lungs from mice injected with B16-F1 cells have been briefly described [5]. Extrapulmonary sites of metastasis include the liver, spleen, adrenals and mesentery lymph nodes [6].

In the present study, the features of the parent tumor are further clarified and compared with those of the F1 variant line using light microscopy. Three possible sites of metastasis are also examined as is the effect of short and long term in vivo growth time on tumor histology.

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MATERIALS AND METHODS

Animals

Male C57BL/6 mice, 22–25 g, were obtained from The Jackson Laboratories, Bar Harbor, Maine. B16 melanoma and B16-F1 cells were supplied by the EG & G Mason Research Center, Worcester, MA.

B16

Twelve mice were etherized for sedation and subcutaneously implanted, by trocar, with approximately 2–4 mm$^3$ of a 15-day melanoma. Two groups of 6 were then established on the basis of the length of in vivo growth time, 14 or 28 days. All melanotic growth from both, as well as liver, lung and kidney tissues from the long term group, were excised, fixed in neutral formalin and carried through routine histological procedure using paraffin embedding and H & E staining.

B16-F1

Twelve mice were sedated and subcutaneously injected (22G needle) with 50,000–65,000 B16-F1 cells. One group of 6 was killed and autopsied after 10 days and another of equal number after 18 days. Melanomas from both groups, as well as liver, lung and kidney tissues from the 18 day group, were excised and prepared for microscopic study.

Measurement

The number of metastases per area of $2.4 \times 10^4$ $\mu$m$^2$ was determined by measuring the radius of the field using a stage micrometer. The percent distribution of cell types was determined by estimating the area occupied by each cell type at 100X. Cell dimensions were determined by measuring approximately 30 cells per mouse.

RESULTS

At 14 days, autopsies of 4 out of 6 B16 implanted mice revealed a small amount of subcutaneous melanoma growth, 0.5–0.75 cm$^3$.

Growth in the B16 group, 28 days post implantation (p.i.), and the B-16-F1 groups, 10 and 18 days p.i., was also present at the subcutaneous site of injection as well as throughout the abdominal mesentery. Half of the mice in the long term B16 group did not survive to 28 days p.i. and were not included in any result analyses or interpretations for the study. B16 tumor growth appeared in 2 of the remaining 3 mice in that group, while both variant divisions had growth incidences of 100% with varying degrees per animal. At both long term in vivo growth times, the extent of melanoma growth was found to be more pronounced. Identical in both lines, the tumor’s gross appearance was soft, smooth, glossy and dark black. Tumor cells comprised 73% of the tumor parenchyma (Fig. 1). Microscopically,
Fig. 1. Tumor cells from a 28-day B16 melanoma. Bar = 40 μm, H & E.

Fig. 2. Tumor cells from an 18-day B16-F1 melanoma. Note variability of pigmentation. Bar = 40 μm, H & E.
they were usually densely packed, well differentiated and occasionally pigmented with black/brown melanin granules of different sizes (Fig. 2). The quantity of pigment present was highly variable. Some tumor cells showed none while others contained intermediate to very high levels. In a few cases, the entire cell was completely filled with pigment. It also appeared extracellularly as isolated granules or large shapeless patches. Tumor cells were either round, oval or irregular in shape with sizes that ranged from 6 to 12 \( \mu m \) in length and 40 to 10 \( \mu m \) in width. Darkly staining nuclei were also polymorphous and were randomly located within the cell body. They tended to be large, 4–7 \( \mu m \) in diameter, with a nuclear-cytoplasmic ratio approaching 1:1 instead of the 1:4 or 1:6 of normal cells. The cytoplasm of the tumor cells was usually characterized by at least one dendritic extension and numerous granular bodies. Erythrocytes and vascular structures comprised 18% of the tumor. Granulated tumor cells were often present within the lumen of capillaries or as a lining along the vessel circumference. Connective tissue elements made up 7% of the tumor and appeared as dense trabecular fibers which penetrated between the sheets of tumor cells and also encapsulated the parenchyma, to varying degrees (Fig. 3). Tumor cells were common within and along the stromal extensions. Round or oval glandular ducts, the remaining 2%, had diameters of 5–30 \( \mu m \) and were randomly distributed throughout the tumor. Adipose tissue, with pigmented tumor cells interspersed, was also present in varying degrees along the 10 day variant tumor periphery.

Fig. 3. 10-day B16-F1 tumor. Arrows indicate connective tissue penetration with incorporated tumor cells. Bar = 40 \( \mu m \), H & E.
Metastases in the parent B16 group appeared in the lungs and kidneys of 2 of the 3 mice and the liver of 1 (Figs. 4 and 5). In the variant group, lung metastasis was seen in 5 of the 6 animals, kidney metastasis in 4 and liver metastasis in all. B16 tumor cells comprised 10–15% of the typical lung and 25% of the typical liver while variant tumor cells comprised only 5% of the typical lung but 35–40% of the typical liver. The extent of kidney metastasis, 5% of the tissue, was the same for both lines. In each case polymorphic tumor cells were so heavily pigmented with black/brown melanin granules that morphological detail was obscured. Pulmonary metastases averaged 10–12 μm in length and 6–8 μm in width and were incorporated throughout all parts of the tissue with the majority in the variant line peripherally based. In the kidney, most tumor cells were limited to the region of the connective tissue capsule with only a few isolated colonies present within the main body of the tissue. Sizes ranged from 3 to 18 μm in length and width although large patches of tumor cells 40–80 μm in diameter were not uncommon. Hepatic metastases were randomly distributed throughout all parts of the liver with averages of 15 in the B16 and 25 in the B16-F1 variant per area of $2.4 \times 10^4 \mu m^2$. However, the majority of tumor cells (50–65%) were present within sinusoids rather than the parenchyma. Sizes ranged from 2 to 15 μm in length and 2 to 10 μm in width.

![Figure 4](image_url)

Fig. 4. B16 pigment granules (arrows) within kidney, 28 days post implantation. Bar = 40 μm, H & E.
Fig. 5. B16 liver metastases (arrow). 28 post implantation. Bar = 40 μm, H & E.

DISCUSSION

The B16 melanoma, syngeneic to C57BL/6 mice, appears to be histologically similar to its F1 variant. Macroscopically, tumor nodules from both lines were indistinguishable. Microscopic observations of the B16 parent tumor confirmed those reported earlier [2,3], although in this study average tumor cells were smaller in diameter. Also, within each tumor, diametrically variable glandular ducts were noted.

Tumor cells from both the parent tumor and its variant line were morphologically the same and comprised about 73% of the parenchyma. They were usually densely packed, polymorphous and variable in the extent of their melanin pigmentation. Nuclei tended to be large and quite random in shape and position within the cell body. The appearance and distribution of other parenchymal elements were also similar for both tumor lines and included erythrocytes and vascular structures (18%), connective tissue elements (7%) and the previously mentioned ducts (2%). Although the length of in vivo growth time was found to be important in determining the extent of tumor growth, the distribution of the tumor's constituent elements remained unaffected by time.

Earlier studies cite B16 metastasis to the lungs, liver and spleen [2], and B16-F1 metastasis to the lungs, liver, spleen, adrenals and mesentery lymph nodes [6]. In most cases, melanoma cells had been introduced by intravenous
or intracardiac injections. In this study, after subcutaneous implantation, lung and liver colonization was confirmed and kidney colonization suggested as well. In the parent tumor and variant line, metastases comprised 5% of the kidney with the majority of tumor cells located within the connective tissue capsule. Hepatic colonization was more pronounced in the variant group, however, where 10—15% more tumor colonies were found. In both lines, the majority were located in sinusoids. The reverse was true for pulmonary invasion where 5—10% more metastases were seen evenly distributed throughout lungs from the B16 parent group.

REFERENCES